REMARKS

The Office Action

Applicant's claim for priority for the "EILDV" claims in USSN 09/029,330 is acknowledged. Claims 25, 28 and 31-36 have been rejected under 35 U.S.C. §112, first paragraph. Claims 31 and 32 remain rejected under 35 U.S.C. §112, second paragraph.

The Specification and Pending Claims

The specification has been amended to include a new Sequence Listing, submitted herewith.

Claims 25, 28, and 31-36 remain pending in this application. All of the pending claims are reproduced in Appendix A, enclosed.

In view of the following remarks, Applicant respectfully submits that the present application is in condition for allowance.

New Sequence Listing Submission

In response to the "Notice to Comply with the Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures" attached to the outstanding Office Action (copy enclosed), Applicant submits herewith a Sequence Listing in computer readable form as required by 37 CFR §1.824. In addition, Applicant submits a substitute Sequence Listing as required under 37 CFR §1.823(a) and a statement under 37 CFR §1.821(f). Applicant requests entry of the paper copy and computer readable copy of the Sequence Listing filed herewith for the instant application.

Rejection of Claims 25, 28 and 31-36 Under 35 U.S.C. 112, First Paragraph

In paragraphs 6-7 of the outstanding Office Action, the Examiner has maintained the rejection of claims 25, 28 and 31-36 under 35 U.S.C. 112, first paragraph, for lack of enablement.

Applicant respectfully traverses this rejection. The claims, as presently pending, are directed to a method for treating diabetes, in a prediabetic mammal, or a mammal having partial β cell destruction, by administering a soluble fibronectin polypeptide, e.g., a fibronectin polypeptide comprising an EILDV motif, a chimeric fibronectin-toxin molecule, or a fibronectin polypeptide comprising an alternatively spliced non-type III connecting segment of fibronectin.

To support this rejection, the Examiner relies, in part, on the statement made in the background of the present application (page 4, lines 17-18) that in the past there has been little success in treating human diabetes. The Examiner states that:

> In the absence of working examples drawn to the invention as claimed, the general teachings of ranges and modes of administration are not sufficient to enable the claims, especially in view of the specific statement in the specification that there has been little success in treating human diabetes on page 4. (emphasis added)

Applicant submits that the statement about the little success in treating diabetes quoted by the Examiner refers to immunosuppressive and/or immunomodulatory agents for treating diabetes available prior to the present application. In particular, pages 3-4 of the background describe the shortcomings of prior art methods and compositions in treating diabetes. Unlike the generally non-specific modalities to treat diabetes described in pages 3-4 of the background, the present invention showed how specific inhibitors of VLA-4-VCAM interactions effectively prevented immune cell destruction of pancreatic islet β cells, and thus could be used therapeutically to treat diabetes. Therefore, the statement relied upon by the Examiner does not apply to the present invention.

The Examiner further contends that:

> Further, even if the working examples were to be drawn to the treatment of insulin-dependent diabetes, the working examples are not drawn to fibronectin but to other agents. It cannot be determined, given the information in the specification, that fibronectin will function as claimed based on information from antibodies and VCAM constructs when it is not clear that the antibodies and VCAM bind the same way to VLA-4, bind at the same docking site, bind with the same affinity or avidity. Further, Applicant has not addressed the issues raised drawn to pharmacokinetics of soluble fibronectin, (b') as previously discussed, and as Applicant suggests, a dosage in the suggested range would lead to the masking and prevention of binding to all VLA-4 expressing cells which would result in a general immunosuppression and it cannot be predicted how a general immunosuppression would treat insulin dependent diabetes, (c') the specification dies not teach methods of determining the appropriate dosages in order to selectively target pathogenic cells, Applicant does not define the term "substantially".

Diabetes, as is the case for a number of immune conditions, involves the migration and activation of immune cells, e.g., lymphocytes, macrophages and dendritic cells, to inflammatory sites. In the case of diabetes, the immune cells typically migrate and destroy islet β cells in the pancreas. Applicant discovered, and provided working examples showing, that blocking the

interaction between the Very Late Antigen-4 receptor VLA-4 ($\alpha 4\beta 1$) and one of its ligands, Vascular Cell Adhesion Molecule VCAM-1 is sufficient to significantly reduce the migration of immune cells to pancreatic islet β cells, thereby preventing the destruction of those cells and delaying the onset of diabetes-like symptoms in a rodent model of diabetes.

Applicant provided working examples showing a reduction of diabetes in rodents using antibodies to VLA-4 and VCAM fusion constructs. In particular, the application includes an example in which the administration of anti-VLA-4 antibody (R1-2) significantly inhibited onset of diabetes in a mammalian NOD mouse model and the residual beneficial results of the treatment were extended as long a two months after cessation of administration of the anti-VLA-4 antibody (R1-2). (See, e.g. pages 11:36-14-14 of the specification). The specification also discloses on page 21 that the experiment described for the antibodies was repeated successfully with the soluble VCAM molecule, i.e., VCAM 2D-IgG.

Applicant has shown that blockade of the VLA-4-VCAM interaction by using an anti-VLA-4 antibody or a VCAM construct was sufficient to delay the onset of diabetes *in vivo*. At the time the present application was filed, fibronectin and VCAM were known to be capable of binding to VLA-4 (see e.g., Wayner et al. (1989) *J. Cell. Biol.* 109:1321-1330). Fibronectin polypeptides provide an alternate method for blocking the VLA-4-VCAM interaction. Once the correlation between inhibition of the VLA-4-VCAM interaction and treatment of diabetes was established, similar results were expected using fibronectin polyeptides.

Fibronectin polypeptides and fragments thereof (e.g., the CS-1 domain of fibronectin) were known inhibitors of VLA-4 activity *in vitro* and in numerous *in vivo* models, including autoimmune, transplant rejection and cancer models. More specifically, the particular sites of fibronectin involved in the interaction with VLA-4 were known to be located in the alternate spliced type IIICS or V region (Guan, J-L et al. (1990) *Cell* 60:53). Two distinct sites were recognized: the CS1 motif, in which the minimally active sequence is comprised of the LDV sequence and the CS5 motif, in which the minimally active site is localized to the REDV sequence (Massia, S. et al. (1992) *J. Biol. Chem.* 267:14019). The interaction of VLA-4 and the fibronectin CS1 peptide was demonstrated to be functionally important in the process of transendothelial migration of a leukocyte subpopulation *in vitro* (Kuijpers, T. et al. (1993) *J. Exp. Med.* 178:279).

Strategies involving the use of antibody and/or CS-1 peptide inhibitors of the VLA-4 interactions with VCAM or fibronectin to inhibit immune cell migration to inflammatory sites

were known at the time the present application was filed, and have been used since then in numerous *in vivo* models. For example, adhesion of T lymphoblastoid cells to the synovial endothelium of rheumatoid arthritis patients has been abrogated using either an anti-α4 integrin antibody or by the CS1 peptide (Elices, M. et al. (1994) *J. Clin. Invest.* 93:405). CS1 peptide has also been shown to decrease lymphocyte migration through high endothelial venule cells (Ager, A. et al. (1991) *Int. Immunol.* 2:921). IDS Synthetic CS1 tetrapeptides, which block VLA-4 binding to fibronectin, have been shown to reduce accelerated coronary arteriopathy in cardiac allograft animal models (Molossi, S. et al. (1995) *J. Clin. Invest.* 95(6): 2601-2610). Similarly, Korom et al. (1998) *Transplantation* 65:854-859 showed that a 25-mer alternatively spliced CS1 variant of fibronectin effectively inhibited the development of chronic rejection in cardiac allograft recipients, and depresses the expression of key T cell- and macrophage-associated cytokines/chemoattractants (see also, Coito, A.J. et al. (1998) *Transplantation Proc.* 30:939-940 (describing the use of a similar 25-mer fibronectin peptide to abrogate acute rejection and significantly prolong cardiac allograft survival). Inhibition of VLA-4-mediated cell adhesion using fibronectin peptides has also been successfully used to inhibit tumor metastasis and invasion. For example, Saiki, I. et al. (1993) *Jpn. J. Cancer Res.* 84: 326-335 showed prolonged survival of mice having liver and lung metastasis of lymphoma or melanoma cells, respectively, using a combination therapy of a fusion polypeptide containing the cell binding- and the heparin binding- domain of fibronectin, in combination with anticancer drugs, such as doxorubicin and mitomycin C. Thus, fibronectin polypeptides and fragments thereof comprising the CS1 binding region starting from 4-mers to 25-mers or longer polypeptides have been successfully used to block cell adhesion involving the VLA-4-fibronectin interaction in numerous *in vivo* models, including autoimmune, transplant rejection and cancer models.

In sum, the discovery in the present case was that blocking the VLA-4-VCAM interaction was sufficient to delay the onset of diabetes *in vivo*. This was unequivocally established in the present application using anti-VLA-4 blocking antibodies and VCAM fusion constructs. The presently pending claims are directed to an alternative method of blocking the VLA-4-VCAM interaction using fibronectin polypeptides. Fibronectin polypeptides were known inhibitors of VLA-4 activity at the time the present application was filed, and could have been effectively used to treat diabetes by following the teachings of the specification.

As to the Examiner's comments pertaining to fibronectin dosages and pharmacokinetics, specific effective dose ranges and modes of administration of these VLA-4 inhibitors are

provided in detail in the instant application (see e.g., page 13, lines 4-26). Working examples describing the effectiveness of these inhibitor dosages are extensively described, e.g., Examples 1-5). Fibronectin polypeptides were known inhibitors of VLA-4 activity at the time the present application was filed, as described above, and could have been effectively used to treat diabetes by following the teachings of the specification. The present application provides that dose ranges of non-antibody (e.g., peptide) inhibitors can be between molar equivalent amounts of the antibody dosages discloses (see page 13, lines 12-13). Furthermore, the instant application provides an example of how one would optimize or determine dosages by monitoring the coating of VLA-4 positive cells by the inhibitors over time after administration at a given dose *in vivo*. These dosages can be extrapolated to fibronectin. The determination of dosage is a routine matter for one of ordinary skill in the art.

The Examiner has presented no evidence that the teachings dosages suggested in the specification would be insufficient to prevent the use of fibronectin peptides, as claimed.

The Examiner further states that "it cannot be predicted how general immunosupression would treat insulin dependent diabetes"... "the specification does not teach methods of determining the appropriate dosages in order to selectively target pathogenic cells..."

Applicant respectfully traverses this portion of the rejection. Unlike non-specific immunosuppressive and/or immunomodulatory agents for treating diabetes, e.g., cyclosporin, known in the prior art to treat diabetes (see background of the specification), the present invention showed that by specifically blocking one interaction, the VLA-4-VCAM interaction, it is possible to prevent immune cell destruction of pancreatic islet β cells. Diabetes involves the migration and activation of immune cells, e.g., lymphocytes, macrophages and dendritic cells, to inflammatory sites. Immune cell migration is mediated by a myriad of interactions of cell surface molecules/counterligands, for example, the combination of LFA-1 and VLA-4, and Mac-1 and VLA-4 on the surface of lymphocytes and macrophages, respectively, and by their counter-ligands ICAM (for LFA-1 and MAC-1) and VCAM and fibronectin (for VLA-4). The present invention selectively targets one pathway. Therefore, Applicant's method is not "general immunosuppression," but selective targeting.

Even when selectively targeting the VLA-4-VCAM pathway, it is possible to adjusting the effective concentration of the fibronectin inhibitor to selectively target pathogenic immune cells. The VLA-4 receptor has been shown to have at least two distinct affinity states, a high and a lower affinity state, depending on the level of activation (see e.g., Jakubowski, A. et al. (1995)

Cell Adhesion and Communication 3:131-142). When present on activated cells, e.g., pathogenic immune cells, VLA-4 shows higher affinity for its ligands, e.g., fibronecin peptides, compared to the affinity displayed by VLA-4 on resting cells. Therefore, by adjusting the effective concentration of the fibronectin inhibitor, it is possible to selectively target those pathogenic cells. As explained above, the present specification provides assays for determining fibronectin dosages. The dosage determination is a routine matter for one of ordinary skill in the art.

On pages 6-12 of the outstanding Office Action, the Examiner newly rejects claims 25, 28 and 31-36 under 35 U.S.C. 112, first paragraph, "because the specification, while being enabling for a prophylactic method of delaying insulin dependent diabetes in an individual with a genetic predisposition for diabetes but no beta cell destruction, does not reasonably provide enablement for a method for the treatment of insulin dependent diabetes comprising administering soluble fibronectin polypeptide to a prediabetic mammal or a mammal having a partial beta cell destruction."

To support this portion of the rejection, the Examiner states that:

> One cannot extrapolate the teaching of the specification to the enablement of the claims because the specification does not teach how to treat insulin dependent diabetes. The *in vivo* data presented is not drawn to treating mammals with diabetes. Examples 1, 3 and 5 are drawn to adoptive transfer experiments wherein spleen cells from NOD mice which have developed diabetes are pretreated with antibodies against VLA-4 or a VCAM construct and then transferred into NOD 8 week old **non-diabetic mice** (emphasis added). The experiments are clearly not commensurate in scope with the claimed invention. Further, even if the claims were to be amended to recite a method of preventing or delaying onset of diabetes (a method to which the specification appears to be drawn), these examples would still not commensurate in scope with the claimed invention because no mammal develops diabetes by the injection of diabetic spleen cells and one would not expect that protection from or delay of onset would be effected by coating those spleen cells with antibodies or VCAM constructs. Example 4 demonstrates the effects of anti-VLA-4 antibody treatment on a spontaneous diabetes model. A review of Figure 6 clearly demonstrates that treatment is begun at four weeks post-partum. The specification clearly teaches that female NOD mice are diabetic at about 13-20 weeks. Clearly, the NOD mice injected were not diabetic.

The above-quoted rejection is respectfully traversed. Applicant provided working examples showing a delay in the onset of diabetes in rodents using antibodies to VLA-4 and VCAM fusion constructs. The application includes an adoptive transfer experiment where the administration of anti-VLA-4 antibody (R1-2) significantly inhibited onset of diabetes in a

mammalian NOD mouse model and the residual beneficial results of the treatment were extended as long a two months after cessation of administration of the anti-VLA-4 antibody (R1-2). (See, e.g. pages 11:36-14-14 of the specification). The specification also discloses on page 21 that the adoptive transfer experiment described for the antibodies was repeated successfully with the soluble VCAM molecule, i.e., VCAM 2D-IgG. Moreover, Example 4 shows similar results to the ones showed in adoptive transfer experiments in a spontaneous diabetes mouse model administered rat anti-mouse VLA-4 antibodies twice weekly for 8 weeks. The onset of diabetes was significantly delayed (12-16 weeks delayed). The experiment in Example 4 was not an "adoptive transfer" experiment, rather, rat monoclonal antibody was directly administered to the subject NOD mice.

The results in Example 4 have been further corroborated by Yang et al., 1994, *PNAS* 91:12604-12608, a copy of which is enclosed. Yang *et al.* corroborated that treatment of neonatal mice with anti-integrin alpha 4 monoclonal antibodies for the first 4 weeks of life led to a significant and long-term protection against spontaneous occurrence of insulitis and diabetes. Thus, the delay in the occurrence of diabetes initially demonstrated in "adoptive transfer" experiments has been corroborated in a spontaneous model for diabetes.

As to the Examiner's comment regarding the scope of the claims in view of the use of non-diabetic mice in Example 4, the presently pending claims are directed to treatment of a prediabetic or a mammal having partial β cell destruction. The terms "prediabetic," "diabetes onset," and "diabetic" are defined in the specification as follows:

> The term "prediabetic" is intended to mean an individual at risk for the development of diabetes disease (e.g., genetically predisposed) at any stage in the disease process prior to overt diabetes or diabetes onset. The term "diabetic" is intended to mean an individual with overt hyperglycemia (i.e., fasting blood glucose levels $\geq$ 250 mg/dL). The term "overt diabetes" or "diabetes onset" is intended to mean a disease state in which the pancreatic islet cells are destroyed and which is manifested clinically by overt hyperglycemia (i.e., fasting blood glucose levels $\geq$ 250 mg/dL). (page 7, lines 19-25 of the specification)

The term "prediabetic," as used in the claims, refers to the treatment of an individual at risk of having diabetes at a stage prior to overt symptoms. Applicant showed a delay in the onset of diabetes in prediabetic NOD mice, i.e., four weeks post-partum and prior to the onset of overt symptoms. Therefore, the pending claims are commensurate in scope with the claimed invention.

The Examiner cites Cohen et al (Autoimmune Disease Models, A Guidebook, Academic Press, San Diego, 1994) to support the proposition that the present specification does not enable a method of treating a mammal having partial β cell destruction. In particular, according to the Examiner, Cohen et al. teach that at 3-4 weeks of age, pancreatic islet β cells appear to be clear of infiltrating immune cells, which seem to localize to the blood vessels of the islets of NOD pancreas. At 6-7 weeks, the infiltrating cells reach the islets surrounding them or accumulating at one pole and that between 10-12 weeks, the infiltrating cells penetrate the islets (p. 150 of Cohen et al.).

This aspect of the rejection is also traversed. Applicant showed that inhibition of the VLA-4-VCAM interaction prevented immune cell recruitment and thereby destruction of pancreatic islet β cells. Applicant's results apply to the treatment of diabetes prior to overt diabetic symptoms, as well as mammals having partial cell destruction, e.g., mammals having ongoing disease.

Successful treatment of ongoing diabetes using VLA-4 inhibitors is confirmed by Yang *et al.* As shown in Yang *et al.*, treatment of NOD mice after the onset of insulitis from 10 to 14 weeks of age with an anti-integrin alpha 4 (VLA-4) antibody resulted in a significant and long-lasting suppression of ongoing, late stage of the disease (*see e.g.*, Yang *et al. supra* at page 12607 and Fig. 5). In this regard, Yang et al. conclude:

> Furthermore, the fact that blockade of integrin α4 was effective in treating an ongoing diabetogenic process suggests that the progression of autoimmune inflammatory destruction of the β islet cells may require continuous recruitment of lymphocytes and/or inflammatory cells from the circulation. (emphasis added)

Thus, the present invention also enables methods of treating ongoing disease. NOD mice 10 to 14 weeks after the onset of insulitis show partial β cell destruction, methods of treating a mammal having partial β cell destruction using VLA-4 inhibitors are fully enabled.

On page 9 of the outstanding Office Action, the Examiner cites Tisch *et al.* (1994) *PNAS* 91:437-438 to support the proposition that:

> In view of the known lack of success in treating insulin-dependent diabetes and the critical requirement of determining whether a treatment can be used to treat an ongoing autoimmune response as taught it cannot be predicted, based on the information in the specification and the art, that the invention will function as claimed.

This aspect of the rejection is also traversed.

Applicant clarifies that the statements about the lack of success in treating insulin-dependent diabetes refer to the Examiner's misinterpretation of the description in the background of the present application of the shortcomings of the prior art methods and compositions in treating diabetes. This statement does not refer to the present invention.

As to the Tisch reference, Applicant submits that this reference relates to the use of antigen-specific immunotherapy, i.e., induction of T cell tolerance by autoantigen immunization, in treating autoimmune disorders. This method is irrelevant to the present application. As described above, the present method is directed to the successful prevention of immune cell recruitment to pancreatic β cells using specific inhibitors of the VLA-4-VCAM interaction. The methods of the invention are completely different to the induction of T cell tolerance reviewed by Tisch *et al.*

With respect to the Examiner's comment on the treatment of ongoing autoimmune response, the Examiner is referred to page 12607 and Fig. 5 of Yang *et al.*, *supra*, which shows suppression of ongoing spontaneous diabetes in NOD mice using anti-VLA-4 antibodies.

On pages 9-12 of the outstanding Office Action, the Examiner relies on Atkinson *et al.* (1999) *Nature Medicine* 5:601-604 and Bowman et al. (1994) *Immunol. Today* 15:115-1120 to support the proposition that efficacy in the treatment of diabetes in the NOD animal model is not predictive of similar results in humans.

This part of the rejection is respectfully traversed. NOD mice are an art recognized animal model for human type I diabetes. Many key features of human type I diabetes are reflected in NOD mice: (1) the development of insulitis; (2) the inheritance of particular major histocompatibility complex (MHC) class II alleles, representing the major component of genetic susceptibility; (3) the transmission of diabetes by hematopoietic cells in bone marrow; and (4) the T cell dependence of the disease pathogenesis. Table 1 below sets out these and other paralleles between the human disorder and NOD. The table is reproduced from Bowman et al. *Immunology Today* 15(3):115-120, 1994, cited by the Examiner to support this rejection.

Table 1. Comparison of insulin-dependent diabetes in humans and NOD mice

Characteristic	Humans	NOD mice
Genetic predisposition (MHC class II linkage)	+	+
Complex polygenic control	+	+
Environmental effects on gene penetrance	Probable	+
Disease transmissible via bone marrow	+	+
T-lymphocyte-driven insulitic lesions	+	+
Leukocyte infiltrates found in other organs	Sometimes	+
Defective peripheral immunoregulation	+	+
Humoral reactivity to β cells	+	+
Endogenous retroviral genes expressed in β cells	-	+
Diabetic ketoacidosis if untreated	+	Mild
Gender Bias	±	+
Successful intervention therapies	Ongoing	+

The Bowman et al. reference further states (at page 19, last paragraph):

> The NOD mouse has provided a model system to study not only the pathogenesis and natural history of a disease that is similar to human IDD, but also a means with which to prevent the disease in humans. (emphasis added)

As outlined above, the NOD mouse model shares a number of important characteristics with human type I diabetes. The disease develops spontaneously and is not accompanied by general immunodeficiency as in some other animal models, e.g., the BB rat. Differences include simultaneous lymphocyte infiltration of salivary glands and other organs, and a strong female predominance. Despite these minor differences, study of mechanisms involved in insulitis, β-cell destruction, and the generation of other immunological disturbances allows hypotheses concerning human type I diabetes to be developed and tested (see, e.g., Lampeter et al., *Diabetologia* 32:703-708, 1989, a copy of which is enclosed as Appendix B).

Regarding the testing of new therapies, the Pozzilli et al., *Immunology Today* 14(5):193-196, 1993 reference, a copy of which is enclosed as Appendix C, states (at page 196, last paragraph):

> "All new therapies aimed at preventing Type 1 diabetes should first be tested on animal models of the disease and the NOD mouse is one of the most appropriate models for this purpose." (emphasis added)

There is no reason to believe that blocking the VLA-4/VCAM-1 interaction in humans would not produce results similar to those in NOD mice.

In view of the foregoing, reconsideration and withdrawal of the rejection of the pending claims is respectfully requested.

Rejection of Claim 36 Under 35 U.S.C. 112, First Paragraph

In paragraph 12 of the outstanding Office Action, the Examiner has rejected claim 36 "under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." In particular, the Examiner states that:

> One cannot extrapolate the teaching of the specification to the enablement of the claims because the specification does not teach how to make the alternatively spliced non-type III connecting segment so that it will function as claimed. It is well known in the art that alternative splicing produces products with different amino acid constituents whereby additions to, truncations or deletion of amino acids of the protein product are produced. However, applicant has not enabled all of these types of modified connecting segments because it has not been shown that these modified proteins are capable of functioning as that which is being disclosed.

Applicant respectfully traverses this rejection. As described above, at the time the present application was filed, the particular sites of fibronectin involved in the interaction with VLA-4 were known to be located in the alternate spliced type IIICS or V region (Guan, J-L et al. (1990) *Cell* 60:53). Two distinct sites were recognized: the CS1 motif, in which the minimally active sequence is comprised of the LDV sequence and the CS5 motif, in which the minimally active site is localized to the REDV sequence (Massia, S. et al. (1992) *J. Biol. Chem.* 267:14019). The interaction of VLA-4 and the fibronectin CS1 peptide was demonstrated to be functionally important in the process of transendothelial migration of a leukocyte subpopulation *in vitro* (Kuijpers, T. et al. (1993) *J. Exp. Med.* 178:279), and have been used since then in numerous *in vivo* models..

In sum, the location of the alternate spliced type IIICS or V region in fibronectin and its importance in mediating fibronectin binding to VLA-4 were recognized in the art prior to the filing date of the present application. Accordingly, the Examiner is respectfully requested to reconsider and withdraw this rejection.

Rejection of Claims 31 and 32 Under 35 U.S.C. 112, First Paragraph

In paragraph 8 of the outstanding Office Action, the Examiner maintains the rejection of claims 31 and 32, which are directed to a fibronectin polypeptide fused to a toxin moiety, for lack of enablement of claim 32, by stating that treatment with such fusion protein

> would result in the destruction of all lymphocytes and macrophages expressing VLA-4 which would result in a general immunosuppression of the prediabetic mammal or the mammal with partial beta cell destruction. It cannot be predicted how a general immunosuppression would treat insulin dependent (type I) diabetes, especially in a prediabetic mammal.

Applicant respectfully traverses the above-quoted portion of this rejection for the same reasons provided above and reiterated below.

The present invention showed that by specifically blocking one interaction, the VLA-4-VCAM interaction, it is possible to effectively prevent immune cell destruction of pancreatic islet β cells. Diabetes involves the migration and activation of immune cells, e.g., lymphocytes, macrophages and dendritic cells, to inflammatory sites. Immune cell migration is mediated by a myriad of interactions of cell surface molecules/counterligands, for example, the combination of LFA-1 and VLA-4, and Mac-1 and VLA-4 on the surface of lymphocytes and macrophages, respectively, and by their counter-ligands ICAM (for LFA-1 and MAC-1) and VCAM and fibronectin (for VLA-4). The present invention selectively targets one pathway. Therefore, Applicant's method is not "general immunosuppression," but selective targeting.

Even among the VLA-4-VCAM pathway, it is possible to adjusting the effective concentration of the fibronectin inhibitor to selectively target pathogenic immune cells. The VLA-4 receptor has been shown to have at least two distinct affinity states, a high and a lower affinity state, depending on the level of activation (see e.g., Jakubowski, A. et al. (1995) *Cell Adhesion and Communication* 3:131-142). When present on activated cells, e.g., pathogenic immune cells, VLA-4 shows higher affinity for its ligands, e.g., fibronecin peptides, compared to the affinity displayed by VLA-4 on resting cells. Therefore, by adjusting the effective

concentration of the fibronectin inhibitor, it is possible to selectively target those pathogenic cells. As explained above, the present specification provides assays for determining fibronectin dosages. The dosage determination is a routine matter for one of ordinary skill in the art.

In view of the foregoing, reconsideration and withdrawal of the rejection of claims 31 and 32 is respectfully requested.

Rejection of Claims 31 and 32 Under 35 U.S.C. 112, Second Paragraph

In paragraph 9 of the outstanding Office Action, the Examiner maintains the rejection of claims 31 and 32 for the same reasons as previously set forth in Paper No. 10, Section 10, pages 10-11.

Applicant respectfully traverse this rejection on the same grounds stated in Applicant's previous Amendment.

Rejection of Claims 25, 28 and 31-36 Under 35 U.S.C. § 112, Second Paragraph

In paragraph 10 of the outstanding Office Action, the Examiner has rejected claims 25, 28 and 31-36 under 35 USC 112, second paragraph, as indefinite "because claim 25, in the preamble, recites a method of treating insulin-dependent diabetes. The claims are confusing because although the preamble recites a method of treating insulin-dependent diabetes, the method steps are drawn to treatment of mammals who do not have diabetes."

Applicant respectfully traverses this rejection. It is clear from claim 25 that the phrase "treatment of insulin dependent (type I) diabetes" refers to a broad range of stages of diabetes, e.g., prediabetic, partial β cell destruction, and ongoing disease. Prediabetic and mammals having partial β cell destruction are included by this phrase. Accordingly, the Examiner is respectfully requested to reconsider and withdraw this rejection.

SUMMARY

The above rejections and objections are either improper or do not pertain to the claims as presently pending and should be withdrawn. The present claims are in condition for allowance.

If a telephone conversation with Applicant's Attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call Applicant's Attorney at (617) 542-5070.

The Commissioner is hereby authorized to charge payment of any additional fees or credit any overpayment to Deposit Account No. 06-1050

Respectfully submitted,
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"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 23, line 23, has been amended as follows:

The adoptive transfer experiment described in Example 1 was repeated with a VCAM-Ig fusion protein (VCAM 2D-IgG) instead of an anti-VLA-4 mAb. VCAM 2D-IgG is a soluble form of the ligand for VLA-4 (VCAM1) which consists of the two N-terminal domains of VCAM1 fused to the human IgGl heavy chain constant region sequences (Hinges, C_H2 and C_H3). The VCAM 2D-IgG DNA sequence and its translated amino acid sequence are shown in [SEQ ID NO: 9] SEQ ID NOs:9 and 16, respectively. Figure 8 illustrates the fusion protein structure. The fusion protein was constructed by recombinant techniques as described below.

Paragraph beginning at page 23, line 34, has been amended as follows:

In order to isolate a cDNA copy of the human IgGl heavy chain region, RNA was prepared from COS7 cells which has been transiently transfected by the plasmid VCAM1-IgGl (also known as pSAB133). Construction of plasmid VCAM1-IgGl is described in PCT patent application WO 90/13300. The RNA was reverse transcribed to generate cDNA using reverse transcriptase and random hexamers as the primers. After 30 min. at 42°C, the reverse transcriptase reaction was terminated by incubation of the reaction at 95°C for 5 min. The cDNA was then amplified by PCR (Polymerase Chain Reaction, see, e.g., Sambrook et al., Molecular Cloning, Vol. 3, pp. 14.1-14.35 (Cold Spring Harbor; 1989)) using the following kinased primers: 370-31 (SEQ ID NO: 10 and 17):

5'TCGTC GAC AAA ACT CAC ACA TGC C
Asp Lys Thr His Thr Cys

which contains a SalI site, and 370-32 (SEQ ID NO: 11):

5' GTAAATGAGT GCGGCGGCCG CCAA,

which encodes the carboxy terminal lysine of the IgGl heavy chain constant region, followed by a NotI site.

Paragraph beginning at page 24, line 33, has been amended as follows:

The plasmid pSAB142 was constructed as follows. cDNA prepared from COS cells transfected with pSAB133 (as described in the previous section) was subjected to PCR amplification using oligonucleotides 370-01 and 370-29. Oligonucleotide 370-01 includes a NotI site and the nucleotides corresponding to amino acids 1 through 7 of the VCAM-l signal sequence (SEQ ID NO: 13 and 18):

5' GAGCTCGAGGCGGCCGCACC	ATG	CCT	GGG	AAG	ATG	GTC	GTG
	Met	Pro	Gly	Lys	Met	Val	Val

Oligonucleotide 370-29 corresponds to the VCAM-1 amino acids 214-219 and includes a SalI site (SEQ ID NO: 14):

5'AA GTC GAC TTG CAA TTC TTT TAC

The amplified DNA fragment was ligated to the vector fragment of pNN03, cleaved by EcoRV.

Appendix A

25. A method for the treatment of insulin dependent (type I) diabetes comprising administering to a prediabetic mammal, or a mammal having partial β cell destruction, a composition comprising a soluble fibronectin polypeptide, in an amount effective to treat diabetes.

28. The method according to claim 25, wherein the fibronectin polypeptide comprises an EILDV motif.

31. The method according to claim 25, wherein the fibronectin polypeptide is a component of a chimeric molecule.

32. The method according to claim 31, wherein the chimeric molecule further comprises a toxin moiety.

33. The method according to claim 25, wherein the mammal is prediabetic.

34. The method according to claim 33, wherein the prediabetic mammal is a human.

35. The method according to claim 25, wherein the mammal has partial β cell destruction.

36. The method according to claim 25, wherein the fibronectin polypeptide comprises an alternatively spliced non-type III connecting segment of fibronectin.